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The functions and unique features of long intergenic non-coding RNA

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Abstract

Long intergenic non-coding RNA (lincRNA) genes have diverse features that distinguish them from mRNA-encoding genes and exercise functions such as remodelling chromatin and genome architecture, RNA stabilization and transcription regulation, including enhancer-associated activity. Some genes currently annotated as encoding lincRNAs include small open reading frames (smORFs) and encode functional peptides and thus may be more properly classified as coding RNAs. lincRNAs may broadly serve to fine-tune the expression of neighbouring genes with remarkable tissue specificity through a diversity of mechanisms, highlighting our rapidly evolving understanding of the non-coding genome.

Long intergenic non-coding RNAs (lincRNAs) are defined as autonomously transcribed non-coding RNAs longer than 200 nucleotides that do not overlap annotated coding genes. lincRNAs share features with the other transcripts of the long non-coding RNA (lncRNA) family and constitute more than half of lncRNA transcripts in humans (TABLE 1). The existence of lincRNAs was first suggested by studies using `tiling arrays` across genomic sequences, which observed pervasive transcription[1,2] from regions with no known coding genes[3–6]. Assessment of chromatin state signatures in murine cell types provided early support for the presence of active transcription units at the putative loci of these transcripts[7]. lincRNAs have been distinguished from the broader lncRNA class of transcripts, as many lncRNAs share sequence with coding loci. Many publications, however, do not distinguish between these two sets of transcripts and group them collectively as ‘lncRNAs’. The accelerating pace with which both intergenic and genic lncRNAs have been discovered and annotated has contributed to an evolving understanding of the functions of the non-coding RNA world[4,8,9].

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By conservative estimates from GENCODE v25 annotations, 51.8% of the human genome is transcribed, but only 1.2% encodes proteins. The discovery of the role of the lincRNA X-inactive specific transcript (Xist) in X chromosome inactivation (XCI) and gene dosage compensation in the early 1990s (REFS [10,11]) was followed in the 2000s by that of HOTAIR, which represses the transcription of HOX family genes[12]. These studies stimulated interest in considering linc-RNA function in specific cellular contexts, cell types, developmental stages and disease[13]. Of the thousands of lincRNAs subsequently annotated by next-generation sequencing technologies, a small fraction have been functionally interrogated and assigned roles in diverse gene regulation processes, organisms and human disease[14,15–26].

In addition to the lack of physical overlap between lincRNAs and protein-coding genes, the distinction between lincRNAs and genic lncRNAs was supported by gene expression analyses, evolutionary conservation patterns and targeted gene disruptions that did not alter adjacent protein-coding genes or genic RNAs. Whether lincRNAs and genic lncRNAs share features is an area of interest. The presence of lincRNAs within gene deserts may weaken the burden of evolutionary conservation, thereby enabling rapid functional diversification of lincRNA loci and resulting in transcripts with fewer mRNA characteristics compared with genic lncRNAs. Alternatively, the separation of lincRNAs from genic lncRNAs may reflect a semantic distinction that is biologically inconsequential[14]. Characterization of potential systematic differences between lincRNAs and genic lncRNAs is an area of active investigation, as is the relationship of these RNAs to gene regulation and enhancer function.

In this Review, we first provide an evolutionary perspective of lincRNAs and discuss its relevance to lincRNA annotation and to understanding their function. We then focus on the emerging unique features of lincRNAs compared with mRNAs and finally discuss the diverse functions of lincRNAs and propose a mechanistic framework for understanding them.

Evolutionary conservation of lincRNAs

GENCODE currently annotates 13,255 lincRNA transcripts arising from 8,598 genes. Despite a notable lack of primary sequence conservation, lincRNAs are not evolutionarily neutral, showing greater conservation than ancient repeat sequences but less than protein-coding genes. Their poor conservation has confounded efforts to predict lincRNA functions across species[17,27,28] but has also helped to highlight focal areas of potential functional importance[29,30].

Compared with protein-coding RNAs and other non-coding RNAs, mammalian lincRNAs have fewer invertebrate orthologues and have undergone rapid evolution[14]. Approximately 5% of mammalian lincRNAs are conserved in zebrafish, and conservation is typically restricted to short polynucleotide stretches[31]. Some mouse and human orthologues, however, have been shown to phenotypically rescue zebrafish lincRNA loss of function, as in the case of the lincRNA cyrano[14], which is crucial for embryonic development, indicating that at least some lincRNAs are functionally conserved across species.

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LincRNAs have functional roles across a spectrum of species. In the budding yeast *Saccharomyces cerevisiae*, lincRNAs participate in various stress responses, including nutrient starvation[32]. In *Arabidopsis thaliana*, lincRNAs are expressed at lower levels than protein-coding genes, but subsets of lincRNAs exhibit tissue-specific and stress-responsive expression patterns[33,34]. *Caenorhabditis elegans* lincRNAs share features with those of other invertebrates and vertebrates. *C. elegans* lincRNAs harbour short conserved regions not subject to the swift sequence evolution of adjacent gene regions. *C. elegans*-specific lincRNA roles are often reproductive and reminiscent of those in yeast[32], including dauer and sperm formation, establishment of male identity and interacting with sperm-specific transcripts[35]. Thus, across invertebrate species, lincRNAs function in metabolic and tissue-specific processes.

The degree of similarity between mouse and human lincRNAs varies between annotation methods. Nearly half of the mouse non-coding transcripts from cDNA libraries map to the human genome. Only 14%, however, have evidence of expressed sequence tags or cDNA indicating the existence of a human orthologous transcript[36], suggesting that the homologous subset is modest[37,38]. Although low conservation does not obviate similar function, it is unlikely that evolutionary clades would independently develop similar functions for such substantial genomic fractions. These findings called into question the conservation of lincRNA functions and motivated the development of alternative annotation methods[14,38]. Categorization of transcripts based on patterns of histone post-translational modifications at the loci that encode them enabled the identification of >1,000 previously unannotated mouse lincRNAs with substantial (>95%) cross-mammalian conservation[7].

Mouse lincRNA knockout models have revealed lincRNAs with perinatal or postnatal lethal phenotypes, as well as growth and organ-restricted developmental defect phenotypes that cannot be analogously studied in humans[39]. The majority of mouse non-coding RNAs are expressed in neuronal tissue and distinct cortical regions[40]. Many have distant chicken and opossum orthologues[41], which, with their expression patterns[42], suggest they have roles in neurogenesis and the tissue-specific tuning of gene expression, although definitive assignment of lincRNA function based on a knockout phenotype is not straightforward because the phenotype could also result from disruption of a gene regulatory element within a lincRNA locus. Murine genetic studies are thus helpful to model human lincRNA biology, although they are limited by both evolutionary divergence and by the experimental challenges of excluding RNA-independent effects. In both murine models and human cell lines, it is generally difficult to determine whether a rescue phenotype observed by providing lincRNA-encoding DNA in *trans* is due to a transcript-dependent effect, the act of transcription itself, effects on other genes or transcripts, inter actions with other proteins, or lincRNA-derived peptides. Furthermore, *trans*expressed lincRNAs capable of rescue may not produce an accurate phenotype if expressed at non-physiological levels or if expressed at an incorrect subcellular location, highlighting the difficulties inherent to assigning mechanisms to lincRNAs.

Mapping of human lncRNA transcripts, including lincRNAs, across species ranging from mice to fish reveals that ~12% have non-human homologues[43]. The orthologous segments span 21–56% of transcript lengths and globally exhibit modest conservation. The fraction of

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identical bases between lncRNAs that are mapped to different genomic regions is lower than that between orthologous coding gene pairs, greater than random genomic regions and similar to syntenic blocks[28]. Greater conservation at lincRNA promoters than at their downstream regions[44] suggests selective pressure for enhancer-like functions[7,27,45], which has indeed been predicted for a substantial subset of lincRNAs[46], termed e-lincRNAs (TABLE 1).

LincRNAs exhibit a range of conservation patterns. Variable conservation at the nucleic acid level may underlie greater conservation at the (secondary or tertiary) structural level. A minority of lincRNAs are highly conserved at both the sequence and the RNA secondary structure level. This subset includes several well-studied molecules, including MALAT1 (REF. [47]), which is under selection to preserve intron–exon organization as well as secondary structure; MALAT1 is stabilized at its 3′ end by a triple helix structure[14]. Other lincRNAs have conservation biased towards their 5′ ends and exhibit rapid changes at their 3′ ends across vertebrates. TINCR, for example, is conserved at its 5′ end across vertebrates. Its 3′ end is conserved in rhesus macaques but not in mice[48]; another well-studied lincRNA with 5′ conservation bias is the MYC proto-oncogene protein-associated PVT1 (REF. [49]). Such a pattern suggests sequence-specific functions may cluster towards the 5′ end and potentially that species-specific functions could derive from the more divergent 3′ end. Other lincRNAs exhibit focal conservation, which may reflect the fact that some genes currently annotated as lincRNAs are actually not non-coding RNAs but in fact contain small open reading frames (smORFs)[29,40]. A subset of lincRNAs harbour conserved smORFs that give rise to polypeptides with biological function[50]. For example, the 90-amino-acid polypeptide small regulatory polypeptide of amino acid response (SPAR) is encoded by a smORF, which is conserved between mice and humans in the lincRNA LINC00961 (REF. [18]). Alternatively, focal conservation could underlie sequence-dependent lincRNA functions such as interactions with other nucleic acids or proteins or translation-linked but peptide-independent activity. Finally, sequence divergence may suggest transcript dispensability, at least for some lincRNAs[19,20], for example, at the mouse *Blustr* locus, where transcriptional activity largely independent of the produced transcript affects the transcription of a neighbouring gene[19].

To summarize, lincRNAs have diverse conservation patterns and have undergone rapid evolution across species. Although they exhibit less cross-species conservation than do protein-coding genes, lincRNAs are not evolutionarily neutral and may have transcript-dependent and/or transcript-dispensable, species-specific functions.

Comparison of lincRNA and mRNA features

LincRNAs differ from mRNAs in their abundance, genomic localization and subcellular localization, as well as in their metabolic profiles, epigenetic regulation and tissue specificity. In this section, we distinguish, where possible, between lincRNAs and genic lncRNAs.

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Abundance, size and genomic localization

The number of lincRNAs continues to expand, with GENCODE v25 annotating 8,598 genes that meet our criteria for lincRNAs (TABLE 1), compared with 19,950 protein-coding genes. Cap analysis of gene expression by the FANTOM5 consortium was recently used to identify 27,919 human lncRNA genes, of which 13,105 are lincRNA genes[46]. Specific lincRNA inclusion criteria included chromatin signatures[7] and distance from adjacent coding genes (to account for unannotated, alternatively spliced transcripts)[27], although a uniform distance cut-off is lacking. lincRNAs are located from several bases to >3 Mb away from the nearest protein-coding gene, at a median distance of 40 kb — 28% are within 10 kb (REF. [28]), and nearly half are >50 kb away, which we define as 'isolated' lincRNAs (TABLE 1). Relative to mRNAs, lincRNA transcripts have fewer exons (an average of 2.9 compared with 10.7), are shorter (average length of 1 kb compared with 2.9 kb)[27,44] and are expressed at a tenfold lower level[44]. The low expression of lincRNAs in whole-organ tissues — that is, tissue samples derived from complex organs containing multiple tissue subtypes — may be driven by cell-type-specific expression, at least in complex tissues[44,51], consistent with lincRNAs having tissue-defining roles.

Subcellular localization

mRNAs are chiefly trafficked to the cytoplasm, where they undergo translation. lincRNAs, by contrast, are more often located in the nucleus than in the cytoplasm, as demonstrated by fluorescent *in situ* hybridization[48] and ribosome profiling[52]. lncRNAs in general have similar nuclear:cytoplasmic enrichment ratios across cell types[27], although genome-wide subcellular (compartment) lncRNA quantification has not yet been published. The nuclear enrichment of lincRNAs may suggest increased stability and function in the nucleus, although evidence for greater cytoplasmic stability[44] and for degradation by the nuclear exosome[53] exist. The latter may account for relative lincRNA enrichment at the chromatin but depletion in the nucleoplasm compared with mRNAs[53]. Recent work proposed a lncRNA classification schema based on RNA metabolic profiles, under the hypothesis that RNA species that share such metabolic profiles may also share functional profiles[54]. In this work, which found lncRNAs to be synthesized less efficiently and degraded more efficiently and to undergo slower splicing than mRNAs, lincRNAs were found to be evenly distributed across broadly defined RNA functional classes, suggesting that neither genomic position nor metabolic profile globally correlate with RNA functional classification[54].

Transcriptional regulation, biogenesis and splicing

RNA polymerase II (Pol II) transcribes approximately 150,000 pre-mRNAs in the human genome, which undergo 5′ capping, splicing and 3′ cleavage and poly-adenylation[55]. To varying degrees, lincRNAs share these processing features, but whereas mRNAs are more robustly co-transcriptionally spliced and polyadenylated, lincRNAs are more often co-transcriptionally cleaved and prematurely terminated (FIG. 1a). Recently, mammalian native elongating transcript sequencing (mNET-seq) was used to profile lincRNAs[53]. mNET-seq surveys genome-wide Pol II density by detecting the phosphorylation status of the Pol II C-terminal domain (CTD) at single-base resolution. Phosphorylation of the CTD on Thr4 is associated with transcription termination and is enriched in Pol II located downstream of

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mRNA polyadenylation sites, but in lincRNAs it is found across entire transcription units. This suggests that whereas Pol II pauses inefficiently at lincRNA promoters, it pauses throughout lincRNA transcription units, resulting in more frequent transcription termination than observed in protein-coding genes[53]. lincRNAs broadly lack the phospho-CTD features of protein-coding genes and therefore are not consistently associated with CTD phosphorylation forms that increase mRNA-like processing[53].

Pol II transcripts can be defined by their positional relationship to coding genes: promoter upstream transcripts in the antisense direction (PROMPTs)[56] and enhancer RNAs (eRNAs)[57] derive from coding gene promoters and enhancer elements, respectively. Most eukaryotic promoters are divergent (bidirectional) and can generate transcripts in both sense (mRNA) and anti-sense directions. Divergent transcription produces approximately 13% of the annotated lincRNAs located within 10 kb of coding gene promoters, of which 65% are within 1 kb of transcription start sites[28]. Divergent transcription is associated with histone H3 Lys56 (H3K56) acetylation and Pol II Tyr1 phosphorylation, is promoted by SWI/SNF chromatin remodellers and is repressed by the RNA deadenylase CAF1 (REFS [17,58,59]), features that may indicate distinct divergent RNA transcription and RNA processing mechanisms.

The binding of nascent transcripts by the spliceosomal U1 small nuclear RNA (snRNA) inhibits the use of alternative polyadenylation signals, thus preventing premature transcript degradation. Bidirectional promoters give rise to asymmetric enrichment in polyadenylation sites (PASs) in the antisense lincRNA transcript and to enrichment in U1 binding sites in the sense mRNA transcript, an asymmetry that would favour premature transcription termination and polyadenylation of antisense lincRNAs, but efficient elongation and splicing of mRNAs[60]. Contradicting the initial descriptions of fewer U1 sites in divergently transcribed lncRNAs than in their promoter-paired mRNAs[60], a more recent study of lincRNAs specifically found comparable U1 binding motif enrichment and polyadenylation motif depletion downstream of the transcription start site — the so-called U1–PAS axis — in lincRNAs and mRNAs[44]. Despite this evidence, lincRNAs are processed less efficiently: they undergo less co-transcriptional splicing and 3′ end cleavage and polyadenylation[53]. They do, however, undergo alternative splicing, with an average of 2.3 isoforms produced per locus[28]. Decreased splicing in lincRNAs compared with mRNAs may be the consequence of weaker internal 3′ splice site signals and less binding by the splicing factor U2AF65 (REF. [44]). The subset of lincRNAs processed most similarly to mRNAs may be more stable than other lincRNAs and have transcript-dependent roles. For example, Xist and Firre, which have well-characterized transcript functions, have greater splice site conservation and are spliced more efficiently than lincRNAs in general[44]. However, on lincRNAs, splicing efficiency does not correlate with ribosomal association[52], suggesting that, unlike mRNAs, efficiently spliced lincRNAs are not better recognized by the translation machinery. The majority of divergently transcribed lincRNAs are spliced in their tissue of maximal expression, but their expression is not highly correlated with that of neighbouring coding genes, as half of the tissue-specific divergent lincRNAs are paired with ubiquitously expressed neighbouring coding genes; this finding implies that lincRNA processing does not invariably influence or reflect nearby gene expression[28].

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In addition to possessing unique splicing features, lincRNAs also influence the splicing of other RNAs by interacting with splicing factors or masking splicing signals[61]. For example, the lincRNAs MALAT1 (REF. [62]) and GAPLINC[63] bind the splicing-associated factor PSF, suggesting that lincRNA-mediated splicing of other genes affects tumour invasion and metastatic capability by regulating oncogene or tumour suppressor activity. An intriguing class of lncRNAs termed 5′ small nucleolar RNA-capped and 3′ polyadenylated (SPAs) were recently implicated in the pathogenesis of Prader–Willi syndrome. SPAs affect the binding of several RNA-binding proteins, including TDP43 and RBFOX2, to their RNA interactors, thereby supporting alternative splicing patterns that are consistent with the disease state[64]. Additionally, principal component analysis of differential lncRNA gene expression and differential splicing in autism has revealed a high correlation between altered expression and splicing activity, again suggesting a role for lncRNAs in modulating splicing activity with functional impact[65].

Stability and degradation

Whereas cytoplasmic mRNAs are often stable, the degree of stability of lincRNAs is less clear. Early work found relative lincRNA instability compared with mRNAs[66], but more recent analyses of lincRNAs paired to mRNAs with similar expression levels identified similar levels of stability for lincRNAs and mRNAs[44], suggesting that mRNAs expressed at relatively low levels that match lincRNA expression levels may be less stable than mRNAs expressed at relatively high levels. Somewhat surprisingly, ribosome footprinting analyses have identified robust cytoplasmic lncRNA–ribosomal association[67]. Over half of lncRNAs are found in the cytoplasm, and 70% of these lncRNAs have over half of their cytoplasmic transcripts associated with ribosomes[52]. Ribosome-associated lncRNAs have a long pseudo-5′-UTR cap structure and lack repetitive sequences. Ribosome engagement stabilizes some lncRNAs, as ribosome stalling following translation inhibition increases the stability of some ribosome-bound lncRNAs[52]. This engagement may additionally reflect the translation of smORFs into functional micropeptides[68]. Overall, cytoplasmic mRNAs are degraded by three main mechanisms: 3′ deadenylation, 5′ decapping followed by 5′-to-3′ exonuclease-mediated decay, and endoribonuclease cleavage. lincRNAs are degraded by these mechanisms but also by independent mechanisms, such as being targeted to the nuclear exosome[17,53].

As they globally lack canonical ORFs and the ORFs that they do have contain premature stop codons, many translated lincRNAs undergo early translation termination and, like mis-translated mRNAs, may undergo nonsense-mediated decay[69]. lincRNAs can also undergo translation-independent destabilization, for example, by microRNAs (miRNAs; the miRNA let-7 destabilizes HOTAIR[70]), RNA-binding proteins (HuR promotes lincRNA decay[71]) and 3′-end processing[72]. It was recently proposed that lincRNAs that undergo sporadic transcription termination are targeted by the pre-miRNA-processing factor DGCR8 for degradation by the nuclear exosome (FIG. 1b), based on evidence that the inhibition of the nuclear exosome stabilizes nucleoplasmic lincRNAs[53]. An orthogonal study found that, following transcription inhibition, lincRNAs and mRNAs have indistinguishable half-lives[44]. Regarding transcript stabilization by HuR, mRNAs are preferentially bound by HuR at their 3′ ends, but lincRNAs are bound equivalently along their transcripts[44], suggesting that

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differential HuR binding patterns do not influence transcript stability. Reconciling the apparent contradiction in lincRNA stability between the different studies may depend on analysing specific lincRNA subsets in greater detail rather than making global class assertions. It is possible that partially transcribed and poorly spliced lincRNAs are degraded by the nuclear exosome, whereas those processed more like mRNAs escape this fate, thus remaining cytoplasmic and stable.

Epigenetic regulation

LincRNAs and mRNAs can positively or negatively regulate the expression of their own genes, or target other genes, by interacting with chromatin-modifying complexes to modulate the epigenetic landscape of chromatin. lincRNA-coding genes and protein-coding genes are similarly globally enriched at their promoters in transcription-activating histone modifications such as H3K27ac, H3K4me3 and H3K9ac[17,27], and many lincRNA genes are characterized by H3K4me3 at the transcription start site and H3K36me3 along the gene body. Conversely, a number of lincRNAs bind EZH2, the catalytic component of polycomb recessive complex 2 (PRC2), which deposits the repressive H3K27me3 modification[73], although the specificity[74] and requirement[75,76] of lincRNA–PRC2 interactions for lincRNA function have recently been called into question. A subset of active lincRNA promoters are enriched in the repressive H3K9me3 modification, here associated with greater tissue specificity rather than differential expression; neither the importance nor mechanism of this modification are currently known[44] (FIG. 1c). In mice, knockout of *Dicer1*, which encodes the miRNA-processing RNase Dicer, decreased lncRNA expression, particularly that of divergent transcripts. This effect is at least partially mediated by the activation by Dicer of oncogenic MYC, implicating miRNA and MYC circuitry in the maintenance of lncRNA expression, independently of mRNA regulation[77]. Reduced lncRNA expression in *Dicer1*-knockout mice was associated with decreased H3K4me3 and H3K36me levels at the downregulated lncRNA loci, suggesting that Dicer has a chromatin-modifying function that maintains lncRNA expression[77]. In yeast, four ATP-dependent chromatin remodelling factors — Isw2, Swr1, Ino80 and Rsc — repress the transcription of a set of antisense lncRNAs to regulate the expression of overlapping mRNAs[78].

Tissue specificity and developmental patterning

LincRNAs often exhibit remarkable tissue specificity and may function to fine-tune the expression of their target genes in a tissue-specific manner. One study performed unsupervised clustering analyses to calculate the tissue specificity expression scores of individual transcripts. By this metric, 78% of lincRNAs were tissue-specific compared with 19% of mRNAs. This result was independent of expression differences between transcripts, as higher scores were assigned to more highly expressed lincRNAs[28]. Using RNA-sequencing (RNA-seq) data of 30 tissues from the most recent data release of the genotype-tissue expression (GTEx) consortium, a median `tau score` of tissue specificity of 0.90 was calculated for lincRNAs, compared with 0.77 for mRNAs (FIG. 2a), with 60.8% of lincRNAs and 29.4% of mRNAs found to be tissue-specific (FIG. 2b). The absolute number of lincRNAs with a distinct expression signature in a single tissue, defined by their tissue enrichment or depletion patterns, revealed an over-representation of tissue-specific lincRNAs in the brain and testis (FIG. 2c). Other studies have similarly found that one-third

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of lincRNAs have testis-specific expression[28]; these lincRNAs may tightly regulate reproductive functions, as do lincRNAs in yeast[32] and *C. elegans*[35]. Recent work raised the possibility that this tissue specificity may reflect the function of nearly half of lincRNAs as e-lincRNAs, thus representing enhancer-specific cell type expression[46].

Protein-coding genes with a lincRNA located within 10 kb are enriched for transcription regulation and developmental patterning functions[28]. Although some lincRNAs demonstrate regulatory relationships with their neighbouring loci[19], the overall expression of lincRNAs in proximity to coding genes is not invariably more correlated with that of their neighbours than is expression of adjacent coding loci[28]. The attempted characterization of lincRNA functions by clustering with expression-matched rather than proximal coding genes has specified lincRNAs with putative tissue-specific functions[28]. Furthermore, trait-associated lincRNAs are preferentially located at the boundaries of topologically associated domains and are frequently derived from evolutionarily conserved enhancer regions. Their expression correlates with protein-coding genes associated with the same traits, suggesting the presence of a shared *cis* regulatory mechanism that involves the modulation of chromatin architecture[79] and that shared regulation and tissue expression may be related to shared function.

In summary, the tissue specificity of lincRNAs suggests that they fine-tune expression of other genes through physical proximity, similar expression patterns or shared phenotypic contributions[28]. Interestingly, the repressive H3K9me3 modification enriched at lincRNA gene promoters correlates with greater tissue specificity, not with lower expression as is the case for mRNAs[44]. Taken together, these data indicate that the specificity of lincRNA expression may promote tissue establishment and maintenance and that lincRNA functions may be intimately linked to those of mRNAs or other non-coding RNAs expressed in the same tissue.

The functions of lincRNAs

As of its latest data release (January 2015), the database lncRNAdb catalogued 156 human lncRNAs with a putative function. This represents a small minority of the thousands of annotated lncRNAs. In this section, we discuss the range of lincRNA functions described to date, which include regulating chromatin topology by both *cis* and *trans* mechanisms, scaffolding of proteins and other RNAs, acting as protein and RNA decoys, regulating neighbouring genes and producing micropeptides. lincRNAs function broadly to tune gene expression by directly affecting nuclear architecture and by sequestering intracellular molecules or promoting their function, as well as more indirectly via the effects of their transcription or translation. The emerging evidence for the existence of smORFs in a subset of annotated lincRNAs highlights the need to revise categorizations of some lincRNAs to coding RNAs. Notable examples of lincRNAs directly implicated in human disease pathogenesis and their potential as biomarkers and therapeutic targets have been reviewed elsewhere[16,80] but are summarized in TABLE 2. Computational and methodological advances facilitating lincRNA annotation and functional characterization are summarized in TABLE 3.

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Chromatin topology

LincRNAs can enforce both stable and repressive chromatin states — that is, those that increase or repress transcriptional activation. They can locally regulate chromatin structure in *cis* as well as inter-chromosomal nuclear architecture in *trans*[16,21,80,81]. *Cis* lincRNA-mediated chromatin interactions include chromatin looping[82] and transcription activation[83] or repression[84] of target genes. *Trans* lincRNA-mediated chromatin interactions are broad and include regulation of co-expressed coding genes by chromosomal looping[82] or by directly binding chromatin-modifying complexes[74] and transcription factors[85].

HOTTIP is a *cis*-acting lincRNA that promotes the expression of the gene *HOXA*. Transcribed from the 5′ tip of the *HOXA* locus, HOTTIP directly interacts at the *HOTTIP* locus with the adaptor protein WD repeat-containing protein 5, which is a component of the myeloid/lymphoid or mixed-lineage leukaemia protein 1 (MLL1; also known as KMT2A) histone lysine methyltransferase complex and, through chromatin looping, targets MLL1 to the *HOXA* locus — which is up to 40 kb away — thereby inducing H3K4 trimethylation and *HOXA* transcription[82] (FIG. 3a). Further demonstrating its *cis* activity, HOTTIP depletion decreases *HOXA* expression, but not the expression of the highly homologous gene *HOXD*[82].

LincRNAs can modulate developmentally regulated genes in *trans*[45]. For example, HOTAIR[12], which is transcribed from the *HOXC* locus, silences *HOXD* as well as genes on other chromosomes[86]. HOTAIR scaffolds PRC2 with the KDM1A–coREST–REST complex, thereby inducing H3K27 trimethylation and H3K4 demethylation[87], respectively, to coordinately repress transcription (FIG. 3a). More recently, the PRC2 dependency of HOTAIR-mediated transcriptional repression has been challenged by evidence that the HOTAIR–PRC2 interaction is dispensable for HOTAIR function[76]. Such findings suggest there is a need to broadly interrogate and revisit prior evidence for the specificity and functional relevance of lincRNA–PRC2 interactions[75].

LincRNAs are themselves regulated by *cis* and *trans* mechanisms. For example, Xist orchestrates XCI in *cis*[10]. It is a 17 kb transcript[88] expressed from the X inactivation centre[89], which is a 500 kb region that also contains the Xist upstream regulatory non-coding RNAs *Jpx* and *Ftx*[90]. In female mammals, Xist is required for the random silencing of one of two X chromosomes, by spreading along and recruiting PRC proteins to the future inactive X chromosome (Xi)[91]. The *Jpx* transcript transactivates Xist on the Xi[92] by titrating CTCF away[93]; conversely, on the active X, Xist is antagonized by its antisense non-coding RNA Tsix. Tsix induces chromatin asymmetry between the X chromosomes, establishing a binary Xist transcriptional state[94,95]. Xist nucleation on the Xi requires binding of the autosomal YY1 transcription factor to YY1-binding motifs at the repeat C domain of Xist[85]. Although Xist is stable and can diffuse in the nucleoplasm when not chromatin-bound, it is not promiscuous in its binding of chromatin, as is evident by the lack of Xist binding to YY1-binding motifs present at autosomes. This suggests that factors other than YYI contribute to the specificity of Xist tethering in *cis*[85]. Xist was recently demonstrated to affect nuclear architecture by directly binding the Lamin B receptor, which tethers Xist to the nuclear lamina, thereby limiting the mobility of Xi to sequester Xist-coated regions from the active X and maintain a repressive gene state[96]. The Xi is partitioned into two repressive

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chromosomal megadomains by the *DXZ4* boundary element, which is required for the establishment of the Xi chromosomal conformation, but not its silencing[97,98].

Recently, lincRNAs that utilize both *cis* and *trans* mechanisms of action were shown to have roles in the three-dimensional organization of the nucleus. The lincRNA Firre, which is required for adipogenesis, is localized to a 5 kb region around its transcription start site and is required for mediating inter-chromosomal binding in *trans* of at least five loci, thereby establishing nuclear compartments that may support its role in adipogenesis[81]. The nuclear matrix protein HNRNPU binds a 156-nucleotide repeat sequence in Firre and is required for the establishment of the multi-chromosome interactions, possibly by anchoring Firre to chromatin to initiate the formation of the nuclear compartment[81].

Scaffolding and modulating the activity of proteins and RNA

LincRNAs may interact with nucleic acids through sequence-complementarity and with proteins through RNA structural elements. Protein scaffolding and modulation by lincRNAs often defines lincRNA function. In the nucleus, lincRNAs regularly scaffold PRC proteins, thereby affecting chromatin accessibility, nuclear architecture and gene expression. The non-coding RNA *RepA*, located in the *Xist* locus, directly binds EZH2, which is the catalytic subunit of PRC2; this scaffolding is required for initiating XCI and Xist spreading[99]. The Xist-bound proteome was recently described by mass spectrometry and implies the existence of a dynamic lincRNA–protein interaction landscape that changes during cellular differentiation[100]. In addition to the scaffolding by HOTAIR of PRC2 and chromatin at the *HOXD* locus[12], more than 20% of lincRNA transcripts (and <2% of mRNAs) interact with PRC2 (REF. [74]), suggesting the existence of nonspecific PRC2 binding of RNA that warrants further investigation[75].

Given their low abundance, particularly in the cytoplasm[44,53], lincRNAs may efficiently and temporarily scaffold multiple proteins or participate in the formation of stable, low-abundance complexes. Cytoplasmic lincRNAs alter the stability[48], degradation[101] and translation status[71] of target mRNAs. For example, TINCR scaffolds the RNA-binding protein staufen1 with epidermal differentiation-promoting mRNAs that bind the TINCR box motif, thereby facilitating their post-transcriptional stabilization and accumulation[48] (FIG. 3b). Whereas in myogenesis staufen1 promotes both mRNA stabilization and decay, in epidermal differentiation its interaction with differentiation-induced RNAs promotes only mRNA stabilization by an unknown mechanism[102]. lincRNA-p21 binds the *JUNB* and *CTNNB1* transcripts and represses their translation; this effect is countered by the binding of lincRNA-p21 by HuR, which recruits the RNA-induced silencing complex (RISC) to destabilize lincRNA-p21 and promote the translation of JUNB and CTNNB1 (REF. [71]) (FIG. 3c).

Protein and RNA decoys

LincRNAs can inhibit protein, mRNA and miRNA activity by their sequestration. The lincRNA Gas5 mimics the glucocorticoid response element (GRE) by forming a double-stranded structure that binds the DNA-binding domain of the glucocorticoid receptor. This interaction prevents the glucocorticoid receptor from binding its target genes and activating

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their transcription (FIG. 3d). Gas5 levels are low in proliferating cells and increase upon growth arrest, during which Gas5 represses GRE-mediated expression of enzymes involved in the rate-limiting steps of gluconeogenesis and glycogenolysis[103]. As part of the stress response to proteasome inhibition, upregulation of the lincRNA NEAT1 promotes the assembly of `nuclear paraspeckles` and the sequestration of transcription factors to control gene expression[104]. MALAT1, which is required for proper splicing, can similarly sequester splicing factors within nuclear paraspeckles to modulate splicing efficiency[47].

Competing endogenous RNAs (ceRNAs) sequester mi RNAs from their mRNA targets, thereby increasing the expression of target mRNAs. The proposed ceRNA mechanism of function holds that some lincRNAs, pseudogene transcripts and `circular RNAs` accomplish this goal by containing multiple miRNA target sites that sequester miRNAs from binding RISC complexes and thus prevent the targeting and degradation of mRNAs[105]. This mechanism is well illustrated by linc-RoR, which maintains stem cell pluripotency. In pluripotent stem cells, linc-RoR sequesters miR-145, thereby promoting the accumulation of the pluripotency factors octamer-binding protein 4 (OCT4; also known as POU5F1), the transcription factor SOX2 and the homeobox protein Nanog, which are miR-145 targets (FIG. 3e). The levels of linc-RoR decrease during differentiation, and miR-145 is released and promotes the degradation of its pluripotency-promoting targets[106]. Similarly, the lincRNA TUG1, which associates with PRC2 in the nucleus, acts as a sponge of PTEN-targeting mi RNAs in the cytoplasm[22], thereby adopting compartment-specific roles.

Regulators of neighbouring transcription

Less than 1% of lincRNAs have been functionally characterized, raising the possibility that many lincRNA transcripts have no intrinsic function. Although large-scale screening for ORFs through CRISPR–Cas9-mediated disruption is efficient, it does not naturally translate to lincRNA screening because most lincRNAs do not function via ORF-derived activity. Alternatively, CRISPR interference (CRISPRi), which employs a nuclease-dead, catalytically inactive Cas9 fused to a protein repressor domain to repress transcription, was recently used to screen 17,000 lncRNA loci across seven human cell lines for functions in cell growth. Impairing the transcription at 449 lincRNA loci affected cell growth; suppression of 89% of these lincRNAs produced phenotypes only in one cell type, in contrast to protein-coding genes, which generally share growth requirements across cell types. Disruption of 14 of these lincRNAs resulted in local transcriptional changes within 20 gene windows surrounding each lncRNA, suggesting that a small fraction of lncRNAs affect transcription locally[20]. This study underscores the importance of broadly interrogating both transcript-dependent and transcript-independent locus functions, as well as the importance of cellular context.

Another recent study explored sequence-dispensable lincRNA roles. Knockout of 5 of 12 loci in mouse cells affected gene expression in *cis*, largely owing to loss of enhancer-like activity from DNA elements in lincRNA promoters. A transcript-sequence-dependent function was dispensable at all loci. At one such lincRNA locus, *Blustr*, transcript length and the level of transcriptional activity correlated with the expression of the upstream *Sfmbt2* locus, but *Sfmbt2* activation did not require Blustr-specific sequences. Furthermore, the

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transcription of both *Blustr* and *Sfmbt2* requires splicing only of the 5′ end of Blustr, implying that the spliceosome at the nascent Blustr transcript participates in *Sfmbt2* regulation (FIG. 4a). Aside from 5′ splice site designation, this is a sequence-independent function associated with general *Blustr* transcriptional activity, which may include cofactor recruitment, altering of chromatin structure and transcription-promoting protein accumulation[19].

Recently, the act of transcription was proposed to be the cause and not the consequence of nuclear conformation changes that affect gene expression[107]. Supporting this model, transcription from the active X chromosome is sufficient to maintain an open chromatin state[108]. lincRNA transcription may alter the epigenetic state at neighbouring coding loci, promoting interactions with nuclear-organizing protein complexes and obviating the requirement for lincRNA accumulation or stability, that is, even low levels of lincRNA transcription could promote the formation of multiple macromolecular complexes — consisting of proteins, nucleic acids, carbohydrates or lipids — without the lincRNAs themselves being in high abundance or comparatively stable. By contrast, macromolecular complex stabilization by lincRNAs would require high lincRNA abundance or stability. These findings underscore the need to perform genetic rescue experiments for any lincRNA with putative functions that are intrinsic to its RNA transcript.

The potential widespread function of lincRNAs as eRNAs has received increasing attention. Because they often do not overlap introns or exons of coding genes, many eRNAs are considered intergenic[109]. eRNAs can act by orchestrating enhancer-directed chromatin looping through `mediator complex` assembly[110,111]. Both PROMPTs and eRNAs may be the consequence of Pol II accumulation at the sites of transcription initiation of coding genes and may therefore not represent classical, independent transcriptional units[53]. In agreement with this, recent 5′ end lincRNA mapping indicated that 45.8% of the analysed lincRNAs originated from enhancers at open chromatin and may thus repre sent an important subclass of eRNAs[46] (e-lincRNAs; TABLE 1). In this regard, lncRNAs encompassing disease-associated `expression quantitative trait loci` (eQTL) are expressed in disease-relevant cell types and co-expressed with their overlapping, eQTL-containing mRNAs; such e -lncRNAs are implicated in regulating the expression of their corresponding coding genes[46].

Proteins such as the transcription factors OCT4, SOX2 and Nanog[112] can regulate their own expression by interacting with *cis*-regulatory elements of their own loci. Because proteins need to be imported to the nucleus from the cytoplasmic sites of protein synthesis in order to directly regulate transcription, the mechanism of protein auto-regulation of transcription is not strictly in *cis*. lincRNAs, by comparison, are produced and can act in the nucleus and are therefore poised to more intimately regulate their own expression or the expression of nearby loci purely in *cis*[17]. A range of lncRNA *cis*-regulatory mechanisms of gene expression not yet described for the intergenic subset were recently reviewed[17]. They include regulation by eRNAs[111], lncRNAs transcribed from imprinted loci[113], autoregulatory lncRNAs and antisense lncRNAs[17]. Aside from antisense lncRNAs, which by definition overlap coding loci, these mechanisms may also be shared by lincRNAs.

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Encoding functional micropeptides

Ribosome profiling with deep sequencing (Ribo–seq)[67,114] and proteomic approaches[115] have enabled the identification of functional smORF-encoded micropeptides of <100 codons in length derived from non-coding loci in flies, mice and humans[115,116]. Non-coding ORF-harbouring loci may constitute up to 10% of the genome, and hundreds to thousands of putative micropeptides originate from genes currently annotated as non-coding[115,117].

The primary sequences of smORFs are less conserved than protein-coding genes but more conserved than introns[50], non-ORF lincRNA transcript regions and lincRNA transcripts as a class. smORF regions are depleted of non-synonymous mutations and lack insertions and deletions, suggesting conservation at the peptide level[118,119]. Putative micropeptide-harbouring lincRNAs are expressed at higher levels and in a less restricted pattern than lincRNAs lacking smORFs[119], implying that their translation or their peptide products may function more broadly than the transcription or transcripts of lincRNAs lacking smORFs. The most comprehensive smORF catalogue was derived from cross-species conservation-based *in silico* smORF predictions[119]. Other studies have reported varying putative lincRNA-derived smORF numbers without applying conservation criteria[50]. As the functional characterization of smORFs is in the early stages of experimentation, their quantification and definitions will be an exciting area of study.

Two polypeptides derived from annotated lincRNAs regulate the function of the SERCA membrane pump to control muscle relaxation: myoregulin and DWORF. Myoregulin is a conserved, transmembrane α-helix micropeptide encoded by LINC00948 and expressed exclusively in skeletal muscle. It directly interacts with SERCA to inhibit calcium uptake in the sarcoplasmic reticulum. DWORF is a human LOC100507537-encoded micropeptide that displaces SERCA-inhibitory peptides to increase sarcoplasmic reticulum calcium uptake and promote myocyte contractility[120]. The NoBody peptide is translated from the LINC01420 lincRNA and interacts with mRNA decapping proteins to control the number of `P-bodies`[118]. SPAR is a peptide conserved between humans and mice and encoded by LINC00961, which inhibits mTORC1 activation and muscle regeneration[18] (FIG. 4b).

Importantly, only a small fraction of conserved lncORFs have peptidomic evidence in mass spectrometry data sets, probably underestimated by the origin of these data sets from only a few cell types[119]. We identified 1,179 lincRNAs that contain 1,432 conserved smORFs, which to date include six lincRNAs that produce six experimentally verified micropeptides. These lincRNAs include RP1-302G2.5 (84 aa product, unnamed); RP11-672F9.1 (40 aa product, unnamed); RP11-451G4.2 (34 aa product, DWORF); LINC00948 (also known as MRLN; 46 aa product, myoregulin); SPAAR (also known as LINC00961; 75 aa product, SPAR); and NBDY (also known as LINC01420; 68 aa product, NoBody). The lincRNAs encoding these smORFs should be more properly categorized as coding rather than non-coding genes[18,68,81,118–121]. Given that more than 80% of the transcript regions of these parental lincRNAs are non-coding — that is, lack translational activity — it is intriguing to speculate that some loci could support both coding and non-coding functions.

The majority of predicted smORF products lack homology with known peptides, which highlights the fact that conservation criteria may not appropriately define smORFs.

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Furthermore, little information exists on the functions of peptides with homologues, thereby necessitating *de novo* peptide characterization, which is not straightforward in the absence of a reliable screen[119]. Chemical biology expertise could greatly contribute to the identification of more micropeptides, the elucidation of their functions and the exploration of micropeptides as drug targets[67].

By chance, any long-enough sequence will contain an ORF, although lincRNAs are defined by their lack of canonical ORFs. Can a lincRNA with coding capacity also have a non-coding function? A fascinating recent example is the switch induced by ultraviolet irradiation from the expression of long, protein-coding *ASCC3* transcripts to the expression of a short non-coding *ASCC3* isoform. The non-coding isoform antagonizes the ASCC3 protein to facilitate transcriptional recovery following ultraviolet-induced damage[24] (FIG. 4c).

Conclusions and future perspectives

How can coding functions evolutionarily replace or exist alongside non-coding functions? Protein-coding genes may be under stringent selective pressure to retain coding capacity. During evolution, some loci may lose coding functions and gain non-coding functions, possibly subsequently to gene duplication. These non-coding loci, including lincRNAs, may regulate three-dimensional nuclear organization[107] and affect gene expression solely by their transcriptional activity and may modulate protein and nucleic acid interactions directly by their transcripts. Some lincRNA loci may undergo additional changes to produce smaller RNAs or regain coding capacity and produce micropeptides[122] that have peptide-dependent or translation-linked functions. In this fashion, a single locus may evolve both non-coding and coding regulatory capacities[123]. The act of lincRNA transcription and translation, as well as the resulting products, may thus evolve to regulate activity at other coding loci[124].

The lincRNA landscape continues to expand and exhibit the diversity of identity and function of lincRNAs. Recent work has demonstrated the dispensability of some lincRNA transcripts for local gene regulation, their role in encoding micropeptides and their implication as enhancer RNAs. Modelling lincRNAs as genetic repositories with the capacity to develop multiple functions, freed from the evolutionary constraints of mRNAs, offers a framework for integrating the diverse functions of the non-coding transcriptome.

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Glossary

Tiling arrays

A method used to probe the transcriptome without prior knowledge of the transcribed loci by hybridizing it to DNA or RNA probes.

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Dauer

An alternative developmental stage in nematodes induced by nutrient starvation and characterized by distinct metabolic and morphological features.

Prader–Willi syndrome

A multisystem, genetic disorder secondary to maternal uniparental disomy of the long arm of chromosome 15, or disruption or deletion of the paternal long arm of chromosome 15; characterized by decreased fetal activity, hyperphagia, short stature, mental retardation and hypogonadotropic hypogonadism.

Pseudo-5′-UTR

The region immediately upstream of the first AUG sequence in a long intergenic non-coding RNA.

Tau score

A rank correlation coefficient used to conduct a non-parametric hypothesis test for the statistical correlation between data variables.

Nuclear paraspeckles

Mammalian nuclear organelles, the formation of which is dependent on non-coding RNA species that may have a role in nuclear retention of translatable RNA.

Circular RNAs

Conserved RNAs formed by pre-mRNA back splicing, the function of which may be linked to that of their host genes.

Mediator complex

A conserved protein complex involved in transcription regulation and with multiple roles in gene regulation.

Expression quantitative trait loci

(eQTL). Genetic sequence variants associated with expression changes in one or more local or distant genes; that is, genetic variants that may account for variable gene expression levels.

P-bodies

Cytoplasmic aggregates of messenger ribonucleoproteins associated with mRNA decay machinery and translation inhibition.

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and non-coding functions of the *ASCC3* locus, with a switch from long, coding transcript production induced by ultraviolet irradiation to produce the short, non-coding RNA product. The non-coding RNA promotes recovery from ultraviolet-induced DNA damage, antagonizing the function of the protein product of the coding transcript. [PubMed: 28215706]

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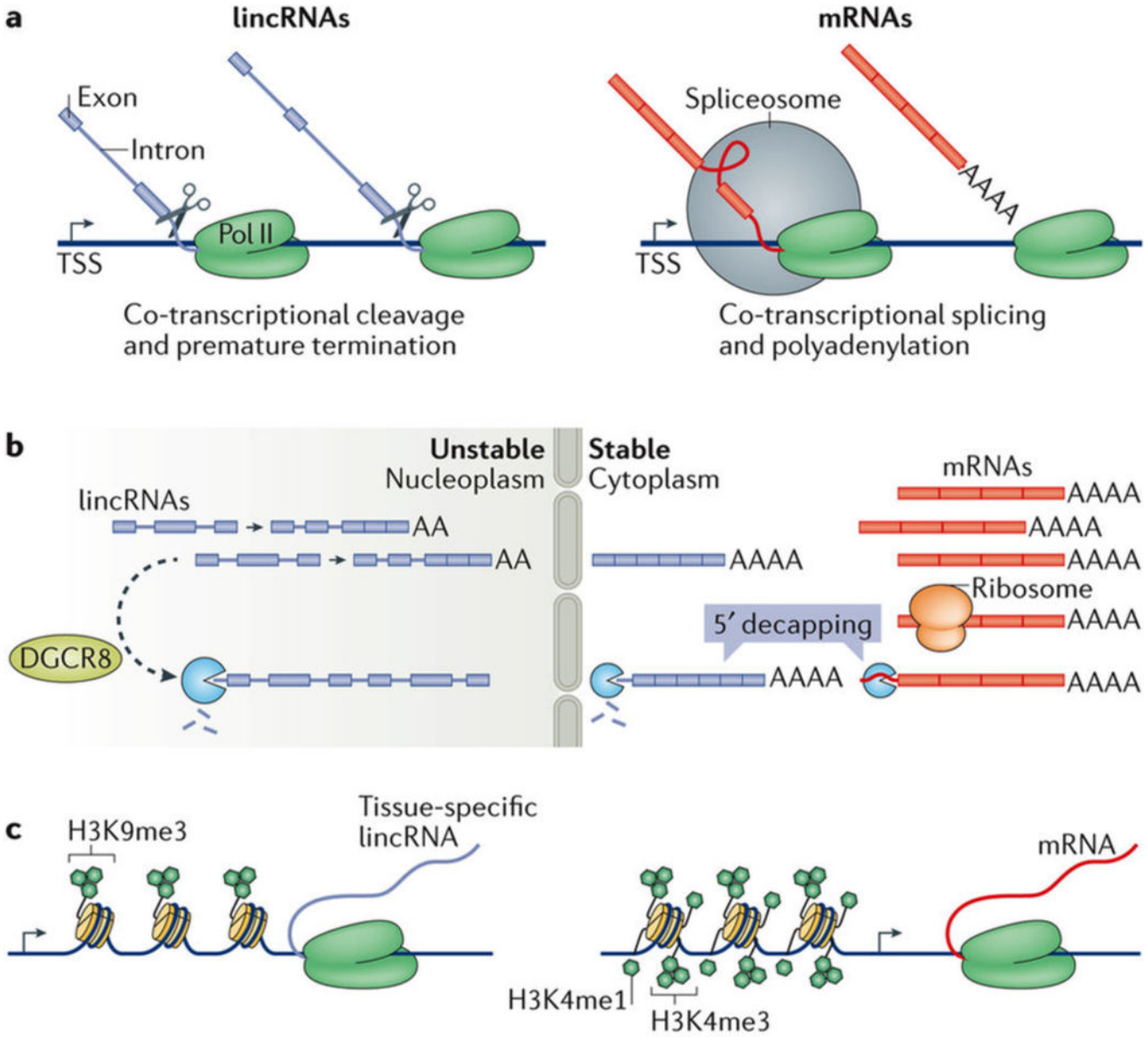

Figure 1. Distinguishing features of long intergenic non-coding RNAs (lincRNAs) and mRNAs

a | During transcription, some lincRNAs undergo cleavage and premature termination, whereas others are spliced and polyadenylated similarly to mRNAs[53]. **b** | LincRNAs are generally more abundant in the nucleus, whereas mRNAs are generally more abundant in the cytoplasm, where they associate with ribosomes. LincRNAs and mRNAs have similar occupancy (residence) at the chromatin, whereas lincRNAs are relatively depleted in the nucleoplasm compared with mRNAs, possibly as a result of degradation by the nuclear exosome[53]. Poorly processed lincRNAs may be targeted to the nuclear exosome for degradation at least partially by microprocessor complex subunit DGCR8, whereas those more similar to mRNAs may remain in the cytoplasm, where they are stable. Cytoplasmic lincRNAs and mRNAs can be degraded following 5′ decapping. **c** | LincRNA-coding and protein-coding genes have globally similar chromatin profiles[7,53]. LincRNAs are

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distinguished by enrichment in histone H3 Lys9 trimethylation (H3K9me3) at their promoters. This is a canonically repressive mark, but in lincRNAs, it is instead associated with greater tissue specificity; the mechanism has not yet been described. H3K4me1 is associated with enhancers and H3K4me3 is associated with promoters. Pol II, RNA polymerase II; TSS, transcription start site.

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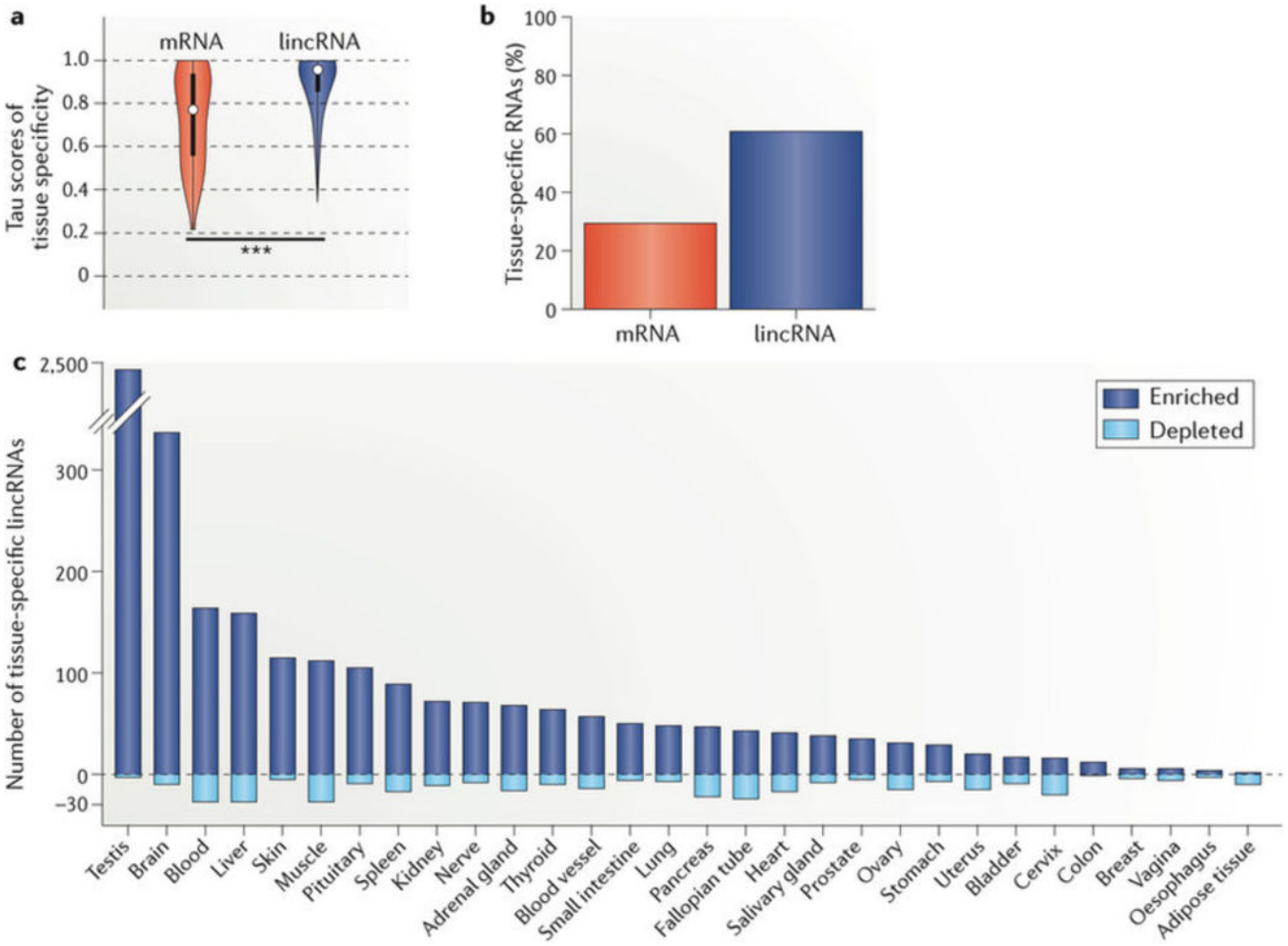

Figure 2. Tissue specificity of long intergenic non-coding RNA (lincRNA) expression

a | We calculated the individual tau (tissue specificity) scores, which range from 0 to 1 (0 for uniform expression; 1 for single-tissue expression), of 7,842 lincRNAs (blue) and 22,285 mRNAs (red) across 30 human tissues from the GTEx Analysis v6 data set (dbGaP Accession phs000424.v6.p1 based on GENCODE v19 (hg19; July 2013)). We additionally calculated tau scores after permutation of each RNA to its assigned tissue to estimate the false discovery rate (FDR). The results are depicted as a violin plot of tau score distributions, demonstrating greater tissue specificity for lincRNAs than mRNAs (median 0.90 for lincRNA; 0.77 for mRNA; 0.47 for permutation; one-sided Wilcoxon test, ***$P < 0.001$). The white dots represent the median; thick and thin bars represent one or two standard deviations from the median, respectively. **b** | Proportion of lincRNAs and mRNAs that are tissue-specific by tau score. **c** | For each lincRNA with significant tissue specificity (tau >0.88; FDR <0.05 by permutation distribution), we re-calculated its tau score 30 additional times, each time excluding one tissue to estimate the individual contribution of the tissue. If tissue specificity no longer reached significance with exclusion of one tissue, we designated the lincRNA as specific to that tissue. Given that such a lincRNA may have tissue specificity through enrichment or depletion, we also calculated the direction of its contribution (one-sided Student's *t*-test, $P < 0.05$). Shown is the number of lincRNAs with a distinct

expression signature in a single tissue owing to enrichment (positive *y* axis) or depletion (negative *y* axis), which highlights the abundance of tissue-specific lincRNAs in the testis and brain.

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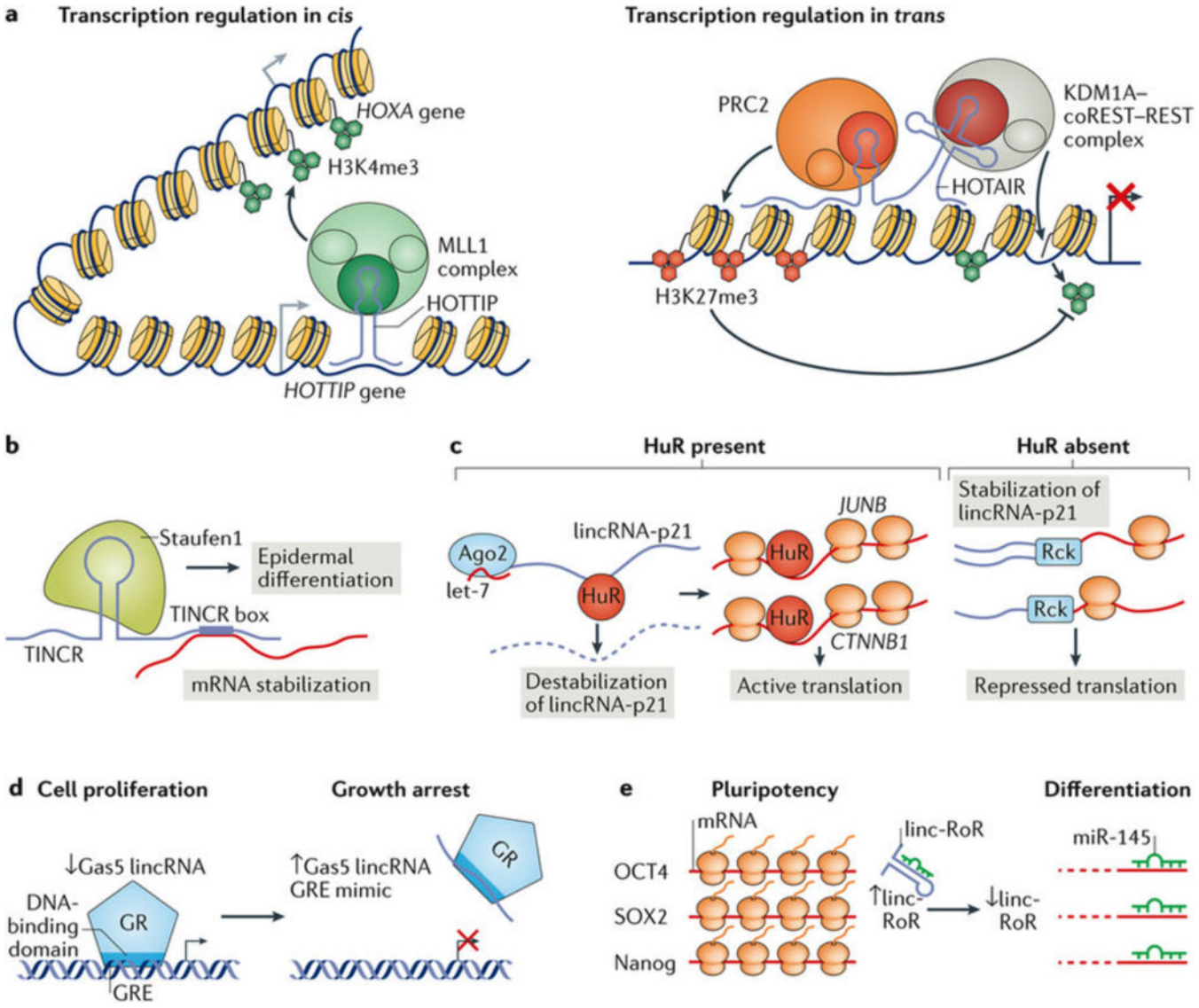

Figure 3. The diverse functions of long intergenic non-coding RNAs (lincRNAs)

a | Regulation of chromatin structure and function in *cis* and in *trans*. HOTTIP associates with the myeloid/lymphoid or mixed-lineage leukaemia protein 1 (MLL1) complex, which catalyses histone H3 Lys4 trimethylation (H3K4me3) to activate *HOTTIP* transcription in *cis* (left). HOTAIR interacts in *trans* with the polycomb recessive complex 2 (PRC2) to mediate its deposition of the repressive H3K27me3 modification and with the KDM1A–CoREST–REST complex to mediate H3K4 demethylation, to coordinate transcription repression at target loci (right)[125]. **b**,**c** | LincRNAs scaffold proteins and RNAs in the nucleus and cytoplasm. **b** | TINCR binds Staufen1 in the cytoplasm, and binds and stabilizes mRNAs through its TINCR box motif to promote epidermal differentiation[48]. **c** | In the presence of HuR, lincRNA-p21 is destabilized by recruitment of the microRNA (miRNA) let-7 in complex with Argonaute 2 (Ago2). HuR association with the lincRNA-p21 target mRNAs *JUNB* and *CTNNB1* results in their translation. In the absence of HuR, lincRNA-p21 remains stable, accumulates and associates with the *JUNB* and *CTNNB1* transcripts in a

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mechanism that is at least partially mediated by co-association with the RNA-binding protein Rck and represses their translation by decreasing their ribosome association[71]. **d**,**e** | LincRNAs act as protein and RNA decoys. **d** | The expression of the lincRNA Gas5 is induced by growth arrest. Gas5 mimics the glucocorticoid response element (GRE) and binds the DNA-binding domain of the glucocorticoid receptor (GR), which sequesters the glucocorticoid receptor from its target genes[103]. **e** | Linc-RoR is abundant in pluripotent stem cells, where it acts as a decoy of the miRNA miR-145, thereby inhibiting the targeting and downregulation of the mRNAs of the pluripotency factors octamer-binding protein 4 (OCT4), the transcription factor SOX2 and homeobox protein Nanog. As linc-RoR levels decrease during differentiation, miR-145 is released and mediates the degradation of its targets to promote differentiation[106]. Part **a** is from REF. 125, Macmillan Publishers Limited.

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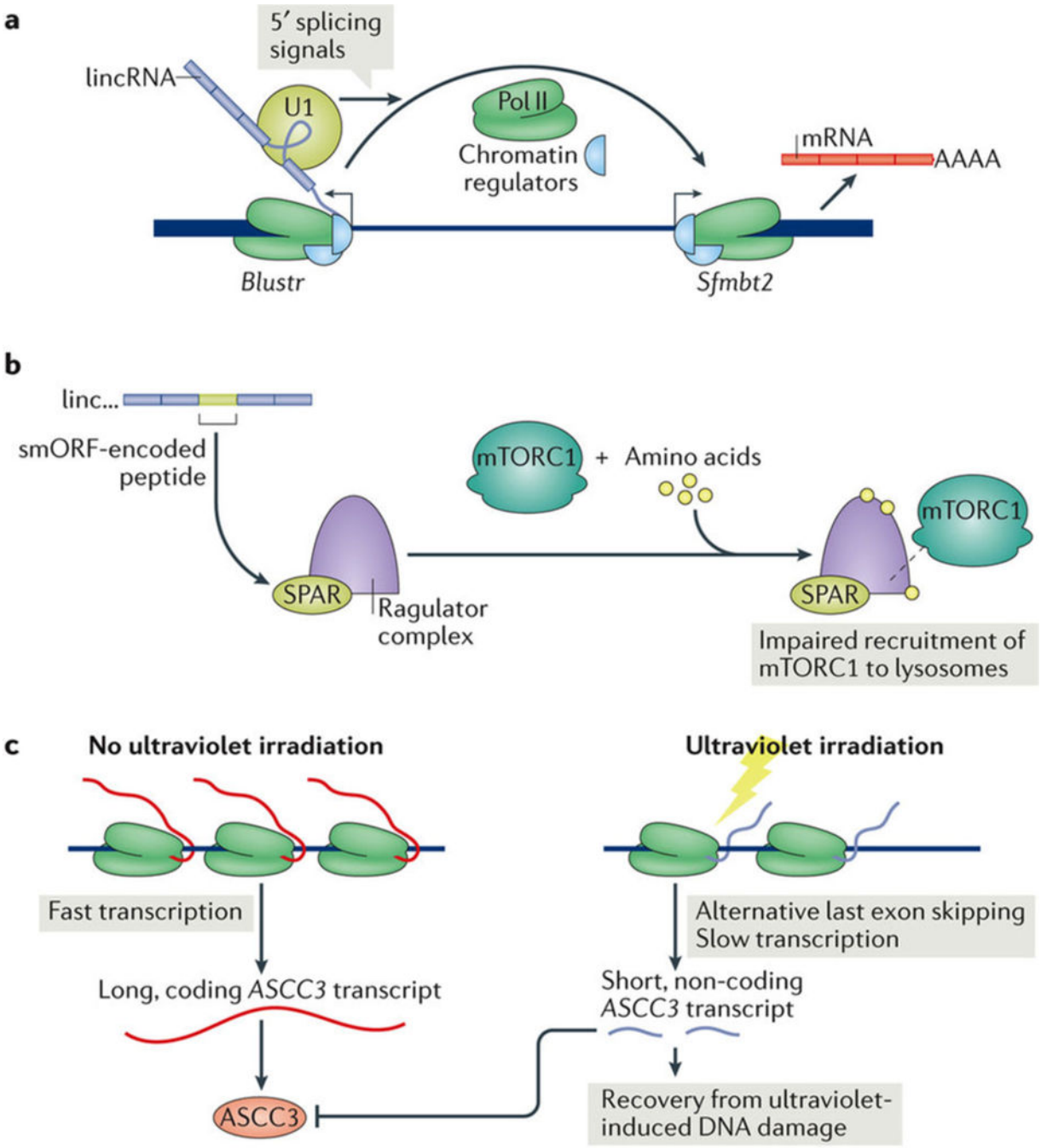

Figure 4. Long intergenic non-coding RNAs (lincRNAs) that function through their transcriptional and translational activity

a | LincRNAs can regulate neighbouring genes by sequence-independent regulatory functions. In mice, splicing of the 5′ end of the lincRNA Blustr as well as general transcriptional activity at the *Blustr* locus activate the transcription of the upstream locus *Sfmbt2*, possibly by recruiting cofactors, accumulation of pro-transcription proteins and chromatin alteration[19]. **b** | Some lincRNAs encode micropeptides. A conserved, small open reading frame (smORF) in the lincRNA LINC00961 produces the peptide small regulatory

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polypeptide of amino acid response (SPAR). In the presence of amino acids, SPAR interacting with the Ragulator complex impairs the recruitment of mTORC1 to the lysosome and thus inhibits mTORC1 activation[18]. **c** | In the absence of ultraviolet irradiation, *ASCC3* is efficiently transcribed to produce a long, coding transcript that produces the ASCC3 protein, which is a component of the ASCC complex that represses ultraviolet-induced DNA damage repair. Ultraviolet irradiation leads to slower transcription at the *ASCC3* locus and induces the use of an alternative, proximal last exon and the expression of a short, non-coding RNA. This non-coding RNA antagonizes the function of the ASCC complex, thereby promoting recovery from ultraviolet-induced DNA damage[24]. Pol II, RNA polymerase II; U1, U1 small nuclear RNA.

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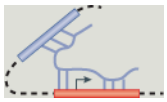
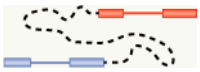
Table 1

Definitions and classifications of lncRNA and lincRNA

RNA	Definition	Number of transcripts in the human genome	Diagram
LncRNA	Autonomously transcribed RNAs longer than 200 nucleotides with minimal coding potential		Key: Blue = lncRNA Red = coding RNA Box = exon Line = intron
Genic lncRNA	LncRNA overlapping a protein-coding transcript at one or more nucleotides	7,169 (of which 1,317 are e-lncRNAs)	
Nested	LncRNA genes contained entirely within protein-coding transcripts	1,256 (S), 2,917 (AS)	
Containing	Protein-coding transcripts contained entirely within lncRNAs	38 (S), 138 (AS)	
Overlapping	LncRNA genes neither ‘nested’ nor ‘containing’	206 (S), 1,867 (AS)	
Multiple relationships	LncRNA with more than one of the above relationships to protein-coding transcripts	747	
LincRNA	LncRNA not overlapping a protein-coding transcript	8,598 (2,488 e-lincRNAs)	
Same strand	LincRNA within 50 kb of and transcribed from the same strand and in the same direction as the nearest protein-coding transcript	2,059	
Convergent	LincRNA within 50 kb of and transcribed head-to-head with the nearest protein-coding transcript	830	
Divergent	LincRNA within 50 kb of and transcribed tail-to-tail with the nearest protein-coding transcript	1,710	
Isolated	LincRNA >50 kb from the nearest protein-coding gene	3,999	

We define long intergenic non-coding RNA (lincRNA) genes as those giving rise to exclusively intergenic transcripts; 663 GENCODE-annotated lincRNA genes give rise to at least one genic transcript and so should be categorized as genic lncRNA genes. We define a lincRNA as ‘isolated’ if it is >50 kb from the nearest protein-coding gene; a specific distance cut-off has not yet been described in the literature. This distance divides the lincRNA class in half between isolated and non-isolated transcripts, with non-isolated transcripts further subtyped by their positional relationship with respect to the nearest protein-coding gene. We intersected GENCODE transcripts with a comprehensive enhancer-derived transcript data set[126,127] to quantify the lincRNA and lncRNA transcripts with enhancer-like functions (e-lincRNAs and e-lncRNAs, respectively). AS, antisense; S, sense.

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Table 2

LincRNAs in human disease and development

LincRNA	Role	Refs
In disease		
MALAT1	• Upregulated in multiple cancers; mutated in bladder cancer • Mutation may contribute to overexpression	25,128
PVT1	• Regulates and co-amplifies with *MYC* at the 8q24 locus • Variants associated with end-stage renal disease susceptibility in diabetes	49,129
LINK-A (LINC01139)	• Lipid-binding oncogene in breast cancer; binds PIP3, interacts with and activates AKT • Promotes AKT inhibitor resistance	26
In development		
Linc-RoR	Establishment and maintenance of pluripotency	130,131
TINCR	Promotion of epidermal differentiation	48
ANCR	Maintenance of epidermal progenitor state	132
BANCR	Melanoma cell migration	133
LincR-Ccr2-5′AS	T cell development	134
ALIEN	Cardiac lineage specification	135,136
PNKY	Neurogenesis	42,137
LincRNA-EPS	Immunomodulation	138
LINC00948; LINC00961	Micropeptide (myoregulin and SPAR) regulation of muscle activity and regeneration	18,121
As biomarkers		
PCA3 (urine PCR)	Greater specificity and positive predictive value than prostate-specific antigen for prostate cancer	139
HULC (blood PCR)	• Upregulated in hepatocellular carcinoma • Overexpressed in gastric, pancreatic and colorectal cancer, hepatic metastasis and osteosarcoma; correlated with poor survival	140,141
HOTAIR (tumour PCR)	• Upregulated in 13 cancers; increases death hazard ratio • Associated with poor outcome in ovarian cancer treated with carboplatin	142,143
MALAT1 (tumour PCR)	Expression correlates with metastasis in early-stage non-small-cell lung cancer	144
As therapeutics (mechanisms and examples)		
Post-transcriptional degradation or silencing by antisense pairing	• LNA GapmeRs: single-stranded, stable antisense oligonucleotides against the target; induce lncRNA degradation • Inhibition of brain-derived neurotrophic factor antisense transcript • Activation of paternal *UBE3A* allele in Angelman syndrome	145–147
Harnessing expression specificity for directed drug delivery	Delivery of diphtheria toxin to ovarian cancer cells under the control of the *H19* promoter	148
Modulating action on target	• LNA displacement of Xist from Xi without altering transcript stability	149,150

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LincRNA	Role	Refs
	• Correction of trisomy 21 gene imbalance by redirecting Xist silencing activity to extra chromosome	

Long intergenic non-coding RNAs (lincRNAs) directly participate in ordered and disordered cellular and developmental processes. In cancer, lncRNA functions derive from copy number alteration, somatic mutation or differential regulation and promote tumorigenesis, metastasis and invasion[16,80,135]. LncRNA cancer[16,80], cardiovascular and renal functions[135] have been comprehensively reviewed; recent work has explored non-coding transcriptome alterations in autism[65]. We highlight several lincRNAs here that illustrate the spectrum of involvement in disease and development. There is emerging evidence for lincRNA variants implicated in depression and cancer, which may affect disease-implicated gene expression and disease subpathways[151–153]. Of lncRNAs, 18% have breast cancer subtype specificity, compared with 10% of protein-coding genes[154], suggesting that their profiling would augment unknown primary tumour assignments and contribute to cancer subtype expression. We additionally provide an overview of lincRNAs as biomarkers and their therapeutic horizons. We direct readers elsewhere for a broader perspective on RNA therapies[155,156]. LNA, locked nucleic acid; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; SPAR, small regulatory polypeptide of amino acid response; Xi, inactive X chromosome.

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Table 3

Profiling lincRNA biogenesis, coding potential and interactomes

Method	Description	Discovery
Protein-centric		
nRIP, nRIP-seq	• nRIP followed by PCR or RNA-sequencing • Antibody-based complex isolation	• Xist, Tsix, RepA[70] and HOTAIR interact with PRC2 (REF. [74]); >20% of lncRNAs interact with PRC2 (REF. [74]) • Novel lncRNA discovery[73]
CLIP CLIP-seq, irCLIP	• Ultraviolet crosslinking followed by immunoprecipitation • In irCLIP, infrared-dye-conjugated adaptors enable reduced input[157] • Lower background than nRIP[158]	Firre interacts with hnRNPU to affect nuclear architecture[81]
RNA-centric		
RNA pull-down	An RNA probe is used to isolate RNA with associated proteins	• Initial description of HOTAIR-PRC2 interaction[12] • LincRNA-p21 interacts with hnRNPK to modulate p53 activity[159]
ChIRP. dChIRP	• Biotin-labelled oligonucleotides are tiled across the entire RNA transcriptome; the RNA is isolated using streptavidin and identified by sequencing, western blotting or mass spectrometry[100,160] • In dChIRP, domain-specific oligonucleotides are used to define the RNA segment involved[161]	• Definition of HOTAIR genome occupancy • HOTAIR nucleates Polycomb domains[160]
CHART	Probes identified using RNase H are used to empirically determine hybridization region	Definition of MALAT1 genome occupancy; enrichment at active genes and for nuclear paraspeckle components[162]
RAP	Oligonucleotide hybridization using long probes (>60 nucleotides); more stable isolation	• Xist depletion at the *FIRRE* locus, suggesting that *FIRRE* escapes X chromosome inactivation[81] • SHARP protein as Xist-specific interactor required for Pol II exclusion from Xi; with HDAC3, required for PRC2 recruitment[163]

With increasing evidence for long intergenic non-coding RNA (lincRNA)-encoded micropeptides, attention has turned towards estimations of small open reading frame coding potential within lincRNAs by sequence conservation[164] or ribosomal profiling[124,165]. Orthogonal approaches to predict effects of non-coding variants using deep convolutional neural networks have been proposed[166]. Multiple methods to characterize lincRNA interactomes have been described; several are highlighted here. CHART, capture hybridization of RNA targets; ChIRP, chromatin isolation by RNA purification; CLIP, crosslinking immunoprecipitation; dChIRP, domain-specific ChIRP; HDAC3, histone deacetylase 3; irCLIP, infrared CLIP; lncRNA, long non-coding RNA; nRIP, native RNA precipitation; nRIP-seq, nRIP followed by sequencing; Pol II, RNA polymerase II; RAP, RNA antisense purification; SHARP, enhancer-of-split and hairy-related protein (also known as BHLHE41); Xi, inactive X chromosome.